

MICROBIAL CHARACTERIZATION OF A MINE SOIL SUBJECTED TO DIFFERENT REMEDIATION TECHNOLOGIES COMBINING ORGANIC AND INORGANIC TREATMENTS AND PLANT CULTIVATION

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1 INTRODUCTION

In Portugal, additional research is needed if technologies based on the combined action of plants and the microbial communities they support within the rhizosphere are to be adopted in large-scale remediation actions (Nabais *et al.*, 2008). Plants growing in abandoned mines are useful to indicate the mineral composition of the soil and they are able to accumulate or exclude toxic metals (Pratas *et al.*, 2005). Taking into account that the mine degraded soils have low concentrations of plant nutrients, it is necessary to apply amendments to ensure plant cover when remediation technologies are present. But soil amendments and the development of a root system might induce shifts in the microbial community structure among the different treatments (Pérez-de-Mora *et al.*, 2006). Moreover, data about the toxic effects of heavy metals on soil microorganisms indicated that heavy metal-sensitive bacteria are probably responsible for the decrease in bacterial activity and the competitive advantage of more tolerant ones resulted in a change in community composition (Díaz-Raviña and Bååth, 1996). Hence, relationships between the soil composition, plant species occurring above-ground and the soil microbial communities have been revealed in many research (Kourtev *et al.*, 2003) providing an important link between above and below-ground processes in terrestrial ecosystems. Soil microbial community structure is increasingly being marketed as ecologically-relevant endpoint and it can realistically be incorporated for assessing the potential risks associated with soil amendment strategies on sustainability of soil ecosystems.

Studies of different remediation technologies with mine soils in Portugal, including amendment materials from farming and industrial sources and the use of native plant species (Guiwei *et al.*, 2008; de Varennes *et al.*, 2009) revealed differential effects of treatments on soil enzymes and microbial respiration, suggesting a change in microbial communities. The information about this fact is scarce and had focused on soil biochemical properties, producing no clear results. Phospholipid fatty acid (PLFA) patterns are sensitive indicators of changes in microbial community structure. This technique has been used to elucidate different strategies employed by microorganism to adapt to changed environmental conditions under wide ranges of soil types, management practices, climatic origins and different perturbations (Zelles, 1999). The present study is the first attempt to characterize, by means of the analysis of PLFA patterns, soil microbial population from a Pb-contaminated mine soil subjected to different remediation technologies including revegetation with native herbaceous species.

2 MATERIALS AND METHODS

The study was performed with a soil from the São Domingos mine in the South of Portugal (Table 1) without plants or with native herbaceous plants (*Briza maxima* L., *Chaetopogon fasciculatus* (Link) Hayek and *Spergularia purpurea* (Persoon) G. Don fil.) combined with the following treatments: control (C); inorganic fertilizer (I, 200 mg N, 125 mg P, 420 mg K and 25 mg Mg kg⁻¹ soil.); polyacrylate polymers, half with K⁺ as counter ion and half NH₄⁺, (P, 0.4% of polyacrylate polymer, 125 mg P, 25 mg Mg kg⁻¹ soil); inorganic fertilizer plus organic amendment (O, 200 mg N, 125 mg P, 420 mg K, 25 mg Mg and 15 g of municipal solid waste compost (MSWC) kg⁻¹ soil); polymer plus organic amendment (PO, 0.4% of polyacrylate polymer, 125 mg P, 25 mg Mg and 15 g of MSWC kg⁻¹ soil). The measurements of PLFA patterns were made on soil samples collected 5 months after the application of treatments.

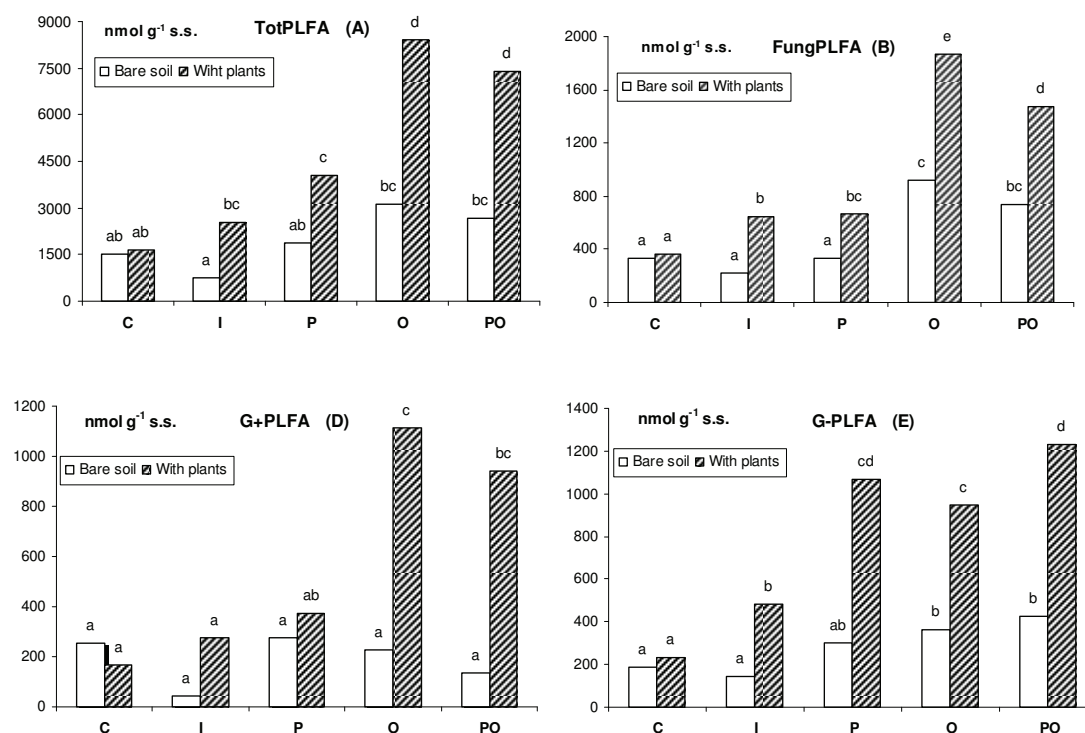
The microbial community structure was determined by phospholipid fatty acid (PLFA) analysis (Frostegard *et al.*, 1993). Fatty acids (PLFA) are designated in terms of the total number of carbon atoms: number of double bounds, followed by the position of the double bound from the methyl end of the molecule. Cis and trans configurations are indicated by c and t, respectively. The prefixes a and i indicate anteiso- and iso-branching; br indicates unknown methyl branching position, 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule; and cy refers to cyclopropane fatty acids. The total microbial biomass (TotPLFAs) was estimated as the sum of all the extracted PLFAs. The sum of the PLFAs considered to be predominantly of bacterial origin was used as an index of the bacterial biomass (BactPLFAs), the quantity of the PLFA 18:2 ω 6,9 was used as an indicator of the fungal biomass (FungPLFA), the PLFAs i14:0, a15:0, i16:0 and 10Me18:0 as indicators of gram-positive (G⁺) bacteria, and the PLFAs cy17:0, cy19:0, 16:1 ω 7c and 18:1 ω 7 as indicators of gram-negative (G⁻) bacteria (Díaz-Raviña *et al.*, 2006). Concentration of all the individual PLFAs data, expressed as mole percentage and logarithmically transformed, was subjected to principal component analysis (PCA) to elucidate the main differences in the PLFA patterns. To compare treatments, data were tested by ANOVA and Tukey's minimum significant difference test was used to differentiate the means.

TABLE 1 Physico-chemical characteristics of initial soil samples

	Texture	pH H ₂ O	C %	N %	C/N	Cu mg kg ⁻¹	Zn mg kg ⁻¹	Pb mg kg ⁻¹	As mg kg ⁻¹
Initial soil	Sandy loam	4.1	0.19	0.03	6.33	91	47	6160	2730

3 RESULTS AND DISCUSSION

The total microbial biomass and the biomass of specific groups (Fig. 1) were significantly greater in soils with plants than in the corresponding bare soils, but the Fungal PLFA/Bacterial PLFA and Gram-negative/Gram-positive bacteria ratios decreased in soils with plants.



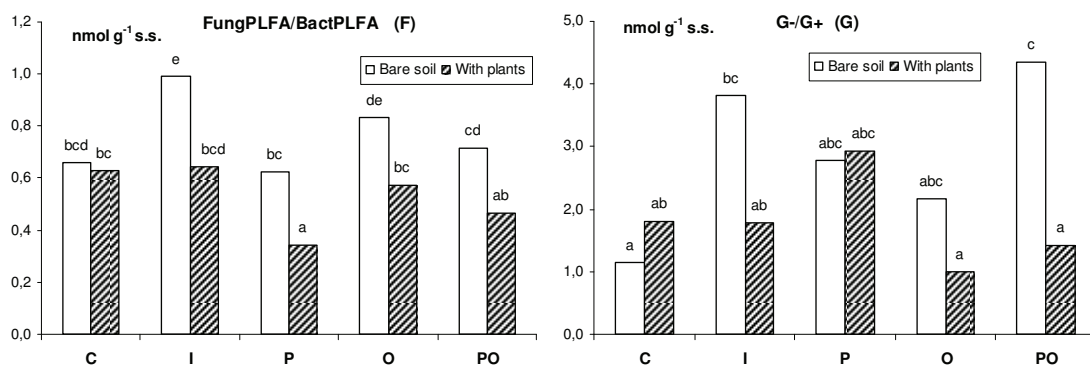


FIGURE 1 PLFA concentrations (mean values of four pot replicates) for the different reclamation treatments: A, total PLFA (TotPLFA); B, fungal PLFA (FungPLFA); D, gram-positive bacteria PLFA (G+ bactPLFA); E, gram-negative bacteria PLFA (G- bactPLFA); F, fungal PLFA to bacteria PLFA ratio (FungPLFA/BactPLFA) and G, gram-negative to gram-positive BactPLFA ratio (G-/G+). Treatments: C, control; I, inorganic fertilizer; P, polymer; O, MSCW; PO, polymer and MSCW. Different letters indicate significant differences at the $P<0.05$ level.

The PCA (Fig. 2) showed that plant cover rather than organic and inorganic treatments plays a dominant role in determining the composition of microbial community. An increase of the branched saturated PLFAs i15:0, i16:0, a15:0 and monounsaturated PLFAs 16:1 ω 9, 16:1 ω 5 and 17:1 ω 8 was detected as consequence of plant cultivation. An effect of different reclamation techniques was also observed; the effect of MSCW was more pronounced than that of the remaining treatments.

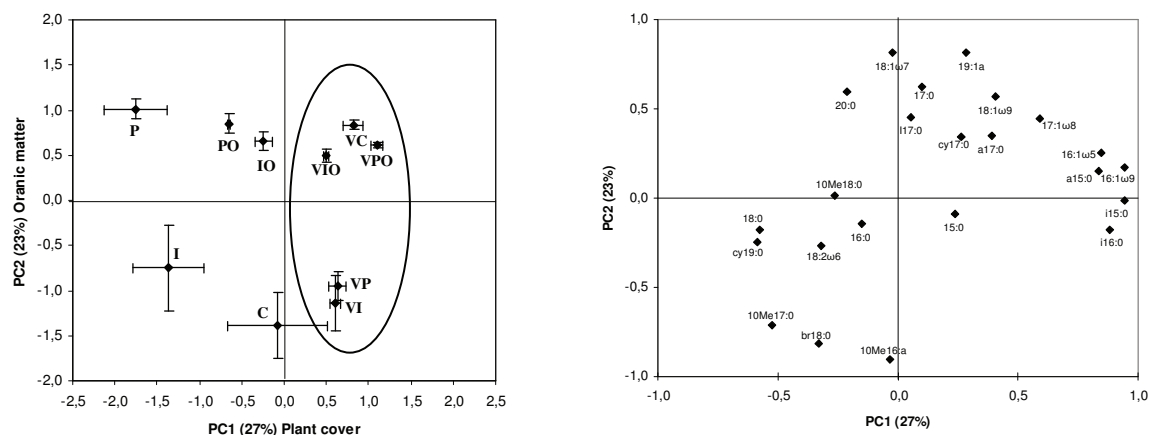


FIGURE 2 Score and loading plots from PCA performed on the PLFAs of the bare soil and soil with plants (V). Treatments: C, control; I, inorganic fertilizer; P, polymer; O, MSCW; PO, polymer and MSCW. Score (\pm SE) and loading plots for the whole data set (four replicates).

4 CONCLUSIONS

The results clearly indicated that a plant cover rather than organic and inorganic treatments played the dominant role in determining the composition of the microbial community, and hence in soil quality of this degraded soil. The sustainability of remediation technologies combining organic treatments and plant cultivation in this degraded mine soil was proved.

REFERENCES

- De Varennes A, Cunha-Queda C, Guiwei Q 2009. Amendment of an acid mine soil with compost and polyacrylate polymers enhances enzymatic activities but may change the distribution of plant species. *Water Air Soil Pollut.* 1573-2932 (Online). DOI 10.1007/s11270-009-0151-4
- Díaz-Raviña M, Baath E 1996. Development of metal tolerance in soil bacterial communities exposed to experimentally increased metal levels. *Appl. Environ. Microbiol.* 62, 2970-2977.
- Díaz-Raviña M, Baath E, Martín A, Carballas T 2006. Microbial community structure in forest soils treated with a fire retardant. *Biol. Fertil. Soils* 42, 465-471.
- Frostegård A, Tunlid A, Bååth E 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl. Environ. Microbiol.* 59, 3605-3617.
- Guiwei Q, de Varennes A, Cunha-Queda C 2008. Remediation of a mine soil with insoluble polyacrylate polymers enhances soil quality and plant growth. *Soil Use and Management* 24, 350-356
- Kourtev P S, Ehrenfeld J C, Häggblom M 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology and Biochemistry* 35, 895-905.
- Nabais C, Gonçalves S C, Freitas H 2008. Phytoremediation in Portugal: Present and Future. In: *Methods in Biotechnology, vol. 23: Phytoremediation: Methods and Reviews*. N. Willey © Humana Press Inc., Totowa, NJ (eds.)
- Pérez-de-Mora A, Burgos P, Madejón E, Cabrera F, Jaekelb P, Schlöterb M 2006. Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. *Soil Biology and Biochemistry* 38, 327-341.
- Pratas J, Prasad M N V, Freitas H, Conde L 2005. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. *Journal of Geochemical Exploration* 85, 99-107.
- Zelles L 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils* 29, 111-129.